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TITLE:

**A REAL-TIME DELAY MONITOR FOR FLOW-SYSTEM
CELL SORTERS**

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Running title: Real-Time Delay Monitor

A REAL-TIME ANALYZER FOR FLAG-CELL SORTERS

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SUMMARY

For optimum performance in cell sorting, it is critical to assure proper timing in the charging of droplets to be deflected. A method for determining the transit delay time in cell sorters has been devised and applied to daily operation in the Los Alamos sorter systems. This delay monitor relies on detection of either scattered or absorbed light from cells in the fluid stream near the point of droplet breakoff.

The technology of droplet-generating cell sorters has made significant advances in the decade since the original cell separator was constructed by Fulwyler in 1965 (3,6). One of the daily problems encountered in the operation of a cell sorter is that of knowing the transit time between the point of measuring the properties of a cell in the flow system and the downstream region at which charging of the droplet containing the cell of interest occurs. In the open-jet type of flow system (1,2), this delay time can be determined simply by counting the equivalent number of surface undulations between the sensing spot and the breakoff droplet. However, in the enclosed type flow chamber (9), this kind of measurement is not feasible, and other methods must be employed to determine the sorting delay time. While most of the commercial cell sorters are based on the open-jet flow system, there are several laboratories (7,8,9) that use enclosed flow chambers.

There are two common methods of determining the sorting delay time in enclosed flow chamber systems. The most straightforward method is to sort standard microspheres at several delay times and then to determine empirically the proper delay time by looking for the best sorting yield. In the second method (6,9), the flow chamber is positioned such that the argon-ion laser beam is focused onto the exit stream. The delay time is then measured as a function of position along the fluid stream by first sensing the cells passing through a volume sensing orifice and then by optically sensing their fluorescence as they pass through the focused laser beam. This yields a calibration curve from which delay times can be determined by subsequent measurement of the droplet breakoff position. However, this calibration is good only at the flow rate used during the measurements, and any changes in flow conditions will render the calibration useless. The new method described in this paper uses a dedicated low-power laser to monitor optically the transit time to the droplet charging region during real time.

MATERIALS AND METHODS

The system which we have routinely employed over the past three years monitors the real-time delay from between the optical signals in the sensing region and light scattered or absorbed by cells in the region of droplet breakup. This system, illustrated in Fig. 1, consists of a helium-neon laser (1 to 2 mW), a spherical lens to focus the laser beam, a photodiode (EG&G type SLD-040B) light detector, and appropriate mounting and positioning hardware. In addition, special electronics are used to mix the amplified monitor pulses, the oscillator signals, and the charging pulses to give monitored waveforms that allow ease of system timing. The focal length of the spherical lens is not very critical to the design, and a 10-cm focal length has generally been used for convenience in mounting the laser and lens in the space available. Lenses with focal lengths of 15 and 20 cm have also been tried with equal success. The laser beam can be focused either on the fluid stream above the charging collar or on the droplet stream below the charging collar. In the case of open-jet flow with no charging collar, it is easy to focus on the point of droplet breakoff. The detector is placed approximately 20 cm behind the fluid stream, and a positioner allows adjustment of the angle of the detector with respect to the laser beam. No collection optics are used. The detector may be positioned very close to the laser beam, where it will be sensitive to absorption, or farther away, where it will sense scattered light from cells in the fluid stream.

In addition to the cell signals, there will be a modulation of the laser beam by undulations in the fluid stream or by droplets. In practice, this modulation represents noise that tends to obscure the cell signals of interest. This difficulty is overcome electronically as indicated by the block diagram in

Fig. 2. In this circuit, part of the output from the piezoelectric transducer (PZT) drive oscillator is mixed out of phase with the monitor signal to null the modulation introduced by the fluid. The light modulation pickup can show some harmonic distortion compared to the sinewave oscillator output, but the nulling circuit nevertheless makes a significant improvement in the quality of monitored signals. The delay signal is next mixed with an attenuated portion of the charging pulse, giving a composite waveform for oscilloscope display. A timing diagram for the signals of interest is shown in Fig. 3. From the timing relationships, the charging pulse delay time is adjusted for proper charging of the droplets containing cells to be sorted.

RESULTS AND DISCUSSION

The monitor detector can be positioned very close to the laser beam for absorption-sensitive measurements. In this mode, the detector is just marginally driven into saturation. The presence of a cell will result in a negative-going pulse from this saturation level. In practice, it is more usual (90% of the time) to position the detector in a light-scatter sensitive mode near an angle of 1° .

One of our early observations with the delay monitor system was that occasionally the delay monitor signals would occur randomly in time instead of being synchronized. This was traced to flow problems occurring in or near the exit orifice of the flow chamber. A partial blockage evidently causes turbulent mixing of the cells and fluid in the exit orifice. There was usually no other evidence of this flow problem either in the optical measurements with the argon-laser or in perturbations of droplet formation.

This technique has been invaluable in diagnosing flow problems that would reduce sorting efficiency before such problems otherwise become apparent. The active real-time delay monitor has proven a useful diagnostic system for both improved ease of operation in setting the delay time, as well as giving early warning of flow-system problems. A variation of this method has been used by Menke et al. (6) to observe fluorescence from cells in deflected droplets as a method for verification of sorting in real time and for showing that the delay time is correct. The disadvantage of this system is that it requires a dedicated argon-ion laser. Measurements of cells in deflected droplets could not be done easily with a helium-neon laser.

A potential use for this system as set up in Fig. 1 is in generation of the charging pulse for minimizing the number of droplets that are charged. In

this scheme, the flow-system measurement would initiate a sort pulse which would be delayed. Then this pulse, in coincidence with the delay monitor signal, would generate the charging pulse. This technique should be useful for both types of flow systems, but especially for the enclosed flow chamber for which a larger number of droplet are normally charged.

Another potential use is in improving separation efficiency. There is experimental evidence that a significant fraction of the cells in the exit stream are positioned in the final neck during droplet formation and charging (7). This results in a large dispersion in the trajectories of the deflected droplets. The delay monitor signal could be used in this situation to determine the relative position that the cell will have with respect to the breaking-off droplet. If it is indicated early enough that the cell will be positioned in the neck of the droplet, then it should be possible to shift the phase of the PZT oscillator fast enough to accommodate the cell well within the droplet.

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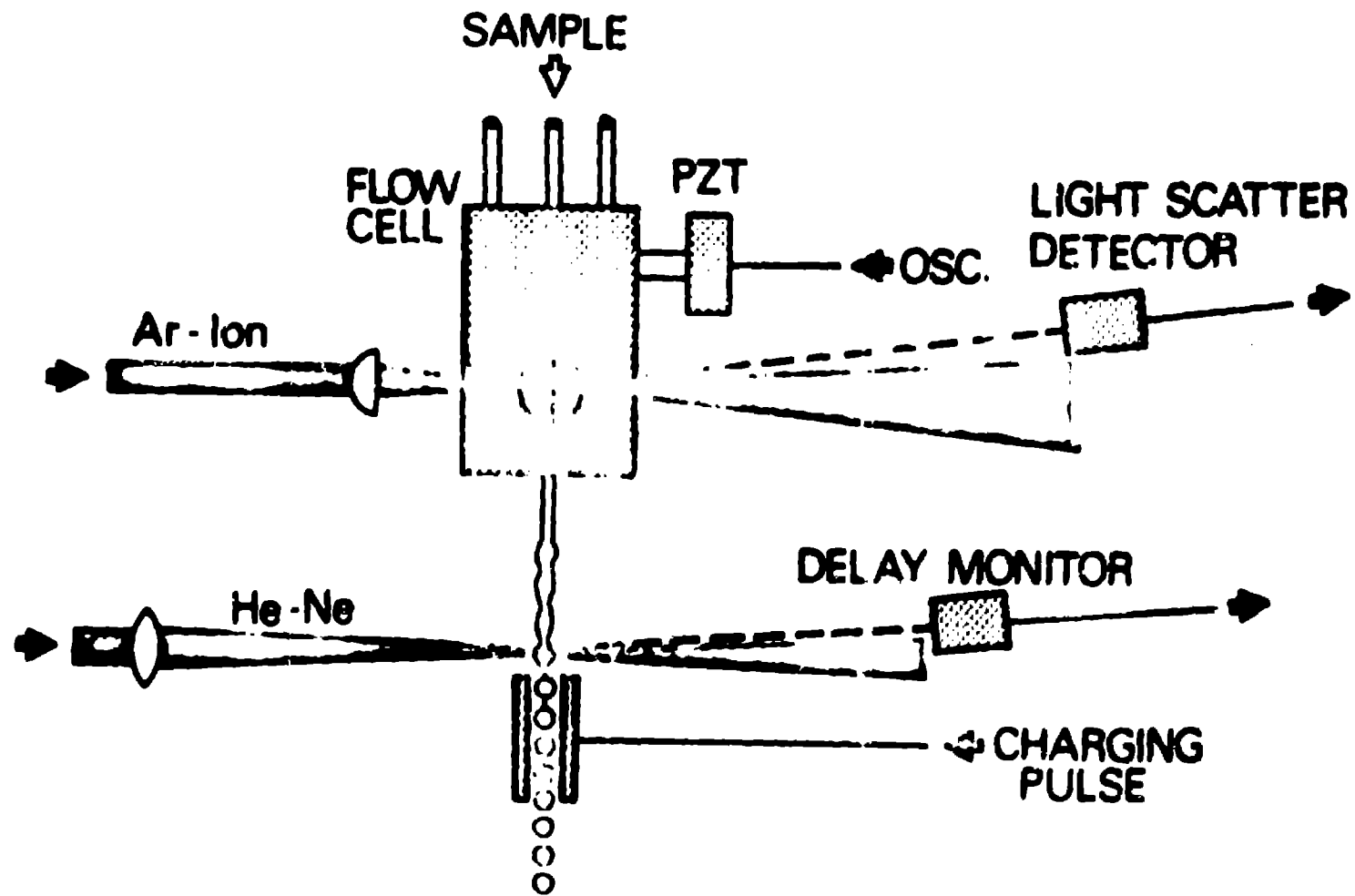
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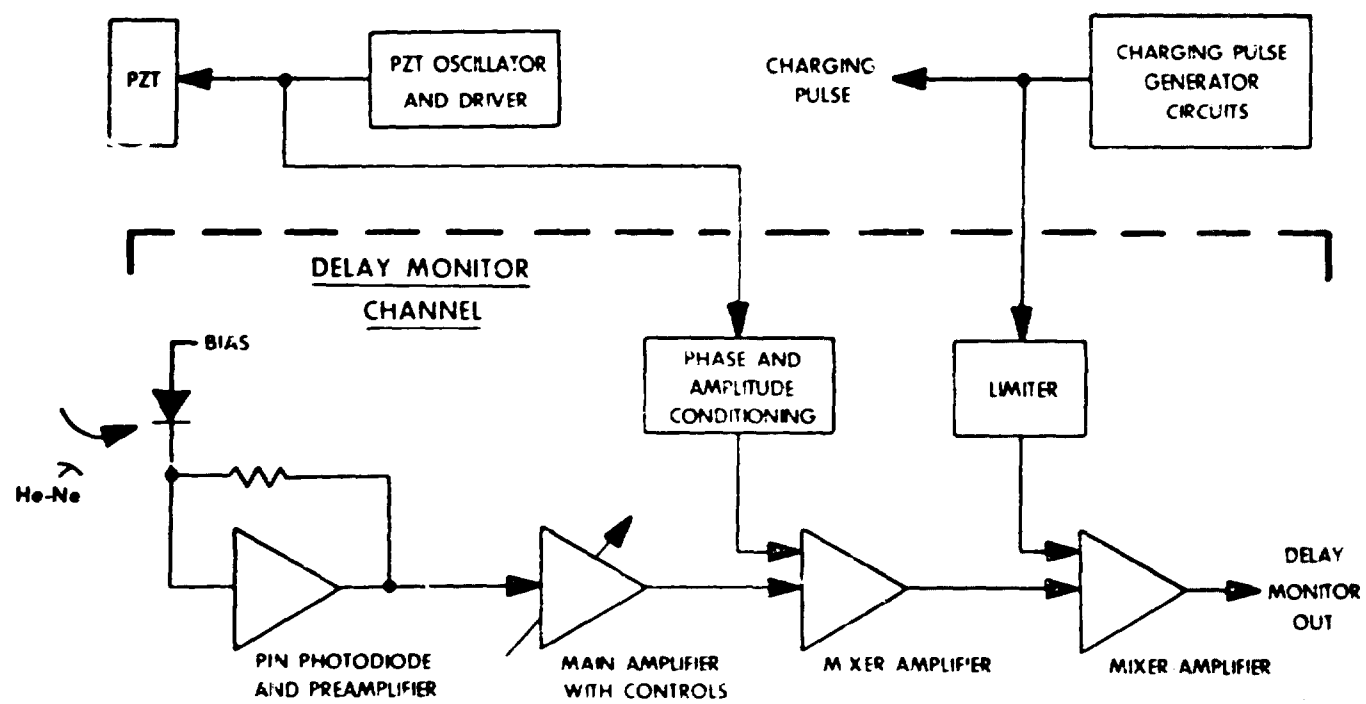
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FIG. 1. Diagram of LASL flow chamber and sorting delay monitor. A light-scatter detector represents the optical measurement of cells passing through the focused argon-ion laser beam. The delay monitor system is shown with the helium-neon laser beam interrogating cells in the exit stream above the charging collar.

SORTING DELAY MONITOR



But, it is not the only one. The other is the "signal" in the
fact that the "signal" is not the same as the "noise" and pre-
sents a "signal" to the "noise" in the "signal".



TIMING DIAGRAM

